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TULAREMIA PROPHYLAXIS: NEW LIVE VACCINE STRAINS

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ABSTRACT

Previous studies on tularemia prophylaxis have established that live vaccine is far superior to killed vaccines for the immunization of animals and man. Further investigation indicated, however, that recovery from tularemia provided the monkey even greater immunity. We postulated that a live vaccine prepared from an attenuated mutant selected from a highly virulent North American strain might afford even greater protection than our present vaccine, which contains a mutant derived from an Old World strain of relatively lower initial virulence. Of the several attenuated mutants of strain SCHU screened for immunogenicity, using the mouse and guinea pig as test animals, SCHU S1-11 offered greatest potential. Live vaccine prepared from this mutant was well tolerated by the monkey when administered intracutaneously or by acupuncture and provided as high a grade of immunity as LVS vaccine against an intradermal challenge of 25,000 cells of highly virulent strain SCHU S4. A subsequent study in man, however, indicated that SCHU S1-11 vaccinees were not more resistant to infection with strain SCHU S4 than LVS vaccinees.

TULAREMIA PROPHYLAXIS: NEW LIVE VACCINE STRAINS

Previous studies on tularemia prophylaxis[1-3] have established that a live vaccine prepared from strain LVS was far superior to killed vaccines for the immunization of animals and man. Further investigation indicated, however, that recovery from tularemia provided even greater immunity.[4] Monkeys* inoculated dermally with the live vaccine were less resistant to challenge than monkeys that had recovered from tularemia initiated via the respiratory route. Table 1 shows that all vaccinees inhaling a challenge dose of 1,000 to 50,000 cells of highly virulent strain SCHU S4 became infected, and 8 of 17 died, whereas fewer monkeys surviving previous infection became reinfected and none died. It was postulated that the difference in the resistance of these 2 groups of animals might be attributable to either or both of two factors: recovery from a more severe infection, particularly as initiated by strain SCHU S4, or recovery from disease produced by a respiratory inoculum.

LVS tularemia vaccine prepared in our laboratories has been evaluated in volunteers by Dr. Samuel Saslaw of the Ohio State University Research Foundation and by Dr. Fred McCrumb of the University of Maryland School of Medicine. Comparable conclusions were made.[2,3] Figure 1 shows some of the results of the study by Dr. McCrumb. Although inoculation with the live vaccine provided increased resistance to respiratory challenges of 200 to 2000 inhaled cells of strain SCHU S4 administered one year later, a dose of 20,000 cells appeared to be overwhelming.

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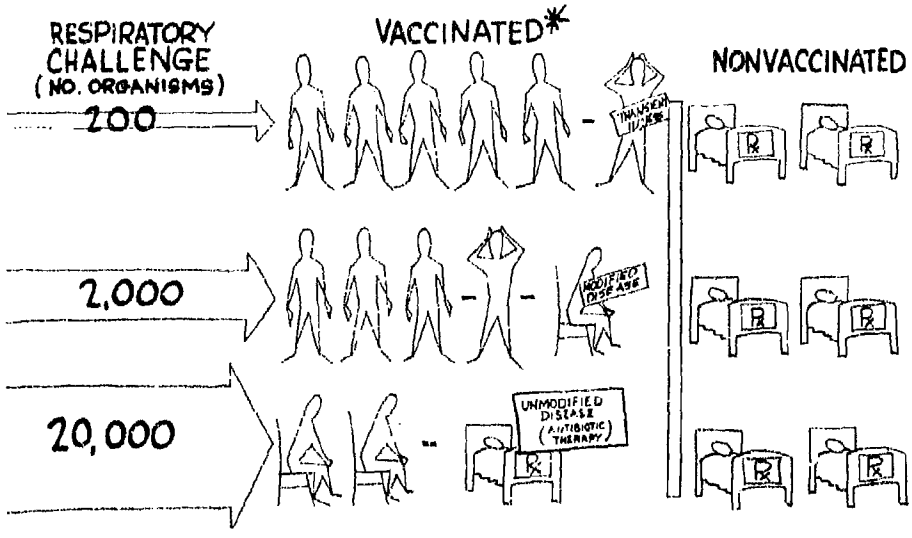
1. Eigelsbach, H.T., and C.M. Downs. 1961. Prophylactic effectiveness of live and killed tularemia vaccines: I. Production of vaccine and evaluation in the white mouse and guinea pig. J. Immunol. 87:415-425.
2. Saslaw, S., H.T. Eigelsbach, H.E. Wilson, J.A. Prior, and S. Carhart. 1961. Tularemia vaccine study: II. Respiratory challenge. Arch. Intern. Med. 107:702-714.
3. McCrumb, F.R., Jr. 1961. Aerosol infection of man with Pasteurella tularensis. Bacteriol. Rev. 25:262-267.
4. Eigelsbach, H.T., J.J. Tulis, E.L. Overholt, and W.S. Gochenour, Jr. 1959. Immunogenicity of live tularemia vaccine for the monkey. Bacteriol. Proc. p. 87 (Abstr.)

* In conducting the research reported here, the investigator adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.



*LIVE TULAREMIA VACCINE

Figure 1. Illness After Respiratory Challenge with Strain SCHU S4.

TABLE 1. SUMMARY OF RESPIRATORY CHALLENGE DATA
(1,000-TO-50,000-ORGANISM EXPOSURES)

Non-Vaccinated		Administered Live Vaccine		Survivors of Previous Infection	
Infected Exposed	Dead Exposed	Infected Exposed	Dead Exposed	Infected Exposed	Dead Exposed
18/18	18/18	17/17	8/17	13/16	0/16
100%	100%	100%	47%	81%	None

This report concerns the screening of several attenuated SCHU mutants for reactivity and immunogenicity in comparison with LVS. We postulated that a live vaccine prepared from an attenuated mutant selected from a highly virulent North American strain might afford even greater protection than our present vaccine, which contains a mutant derived from an Old World strain of relatively lower initial virulence. Colony-type variants appearing on a deficient medium or medium containing deuterium oxide were screened for residual virulence, using the mouse and guinea pig as test animals. Several mutants of reduced virulence were selected for further study.

Table 2 indicates the virulence of the various strain SCHU mutants for the white mouse. Data on vaccine strain LVS and on strain JAP H, a derivative of an Asiatic strain, are also presented. It has been assumed that if a total of approximately 30% of the the animals die, in a titration similar to this but with a dose level of 10^9 included, the strain is sufficiently invasive to elicit immunity in man. Appreciably higher or lower values indicate too virulent or too attenuated a strain for the preparation of live vaccine. LVS, S1-11, and S2-3 appear to meet this requirement, but the rest are somewhat lower in virulence. When survivors were challenged subcutaneously with 1000 cells of SCHU S4 (Table 3), it appeared that LVS, JAP H, S1-11, and S2-3 had provided high-grade immunity at all dose levels; DT and S1-1 vaccine gave less protection, especially at the lowest dose level.

TABLE 2. VIRULENCE OF POTENTIAL LIVE TULAREMIA VACCINE STRAINS FOR THE WHITE MOUSE

Dose[a]	Mortality After Subcutaneous Inoculation, %					
	LVS	JAP H	S1-11	DT	S2-3	S1-1
10^8	48	3	45	22	33	3
10^5	23	0	33	3	13	0
10^4	10	0	28	5	5	0
10^3	20	3	8	0	5	0

a. 40 animals per dose level.

TABLE 3. IMMUNOGENICITY OF VARIOUS PASTEURELLA TULARENSIS STRAINS FOR THE WHITE MOUSE

Vaccine dose	Survival of Vaccinees After Subcutaneous Challenge,[a] %					
	Control	LVS	JAP H	S1-11	DT	S2-3	S1-1
10^8		100	85	100	45	100	49
10^5		97	90	100	23	94	20
10^4		92	85	93	28	95	17
10^3		91	74	100	5	92	7
None	0[b]						

a. 1000 cells of strain SCHU S4.
b. 40 animals inoculated; no survivors.

Virulence titrations of the various strains and mutants in the guinea pig are shown in Table 4. Strains LVS and S1-11, although comparable in virulence for the mouse, were quite different in virulence for the guinea pig; S1-11, DT, and S2-3 caused fatal disease in this animal in contrast to the other strains. Little useful information on comparative immunity was obtained because so few S1-11 and DT vaccinees were available for challenge. However, on the basis of all data available LVS, S1-11, and S2-3 were selected for comparative evaluation in the monkey. Approximately 10^6 cells of the strain to be evaluated were administered intradermally to two Macaca mulatta (Table 5). All strains were innocuous for monkeys and

all vaccinees developed agglutinins for Pasteurella tularensis. Sixty days after vaccination the animals were challenged intradermally with 25,000 cells of strain SCHU S4. Animals that had been vaccinated with LVS or S1-11 did not become febrile or were febrile for only 24 to 48 hours, whereas S2-3 vaccinees and nonvaccinated animals were febrile from the second day after challenge until death or for at least 10 days. Subsequent to this test in the monkey, LVS and S1-11 were used to prepare lyophilized vaccines.

TABLE 4. VIRULENCE OF POTENTIAL LIVE TULAREMIA VACCINE STRAINS FOR THE GUINEA PIG

Dose[a]	Mortality After Subcutaneous Inoculation, %					
	LVS	JAP H	S1-11	DT	S2-3	S1-1
10^8	0	0	100	100	100	0
10^6	0	0	100	100	10	0
10^4	0	0	40	40	0	0
10^2	0	0	30	10	0	0

a. 10 animals per dose level.

TABLE 5. IMMUNOGENICITY OF POTENTIAL LIVE TULAREMIA STRAINS FOR THE MACACA MULATTA

Vaccine Strain[a]	Animal Number	Agglutinin Titer when Challenged	Animal Febrile on Indicated Day After Intradermal Challenge[b] 1	2	3	4	5	6	7	8	9	10
LVS	11	1:80	0	0	0	+	0	0	0	0	0	0
	7	1:80	0	+	+	0	0	0	0	0	0	0
S1-11	64	1:320	0	0	0	0	0	0	0	0	0	0
	15	1:160	0	0	0	0	0	0	0	0	0	0
S2-3	25	1:20	0	+	+	+	+	+	+	Dead		
	16	1:80	0	+	+	+	+	+	+	+	Dead	
None	66	0	0	+	+	+	+	+	+	+	Dead	
	46	0	0	+	+	+	+	+	+	+	+	+

a. 10^5 cells administered intradermally.
b. 25,000 cells of strain SCHU S4.

The reactivity and immunogenicity of LVS and S1-11 lyophilized vaccines were compared in the mouse, guinea pig, and monkey. Monkeys were vaccinated with rehydrated vaccine by acupuncture as used to administer smallpox vaccine. With the exception that the S1-11 vaccine was more virulent for the guinea pig than LVS vaccine, no appreciable difference was observed. Comparable innocuous vaccine lesions were produced in the monkey, and both groups of vaccinees exhibited high-grade immunity against an intradermal challenge of 25,000 SCHU S4 cells. Results on comparative vaccine effectiveness were similar to those obtained in the previous study in which monkeys were vaccinated intracutaneously before a comparable challenge.

After additional studies, including the determination of the sensitivity of S1-11 to antibiotics, S1-11 live vaccine was administered dermally by acupuncture to 18 male adult volunteers. Two of the men developed axillary buboes that required drainage and antibiotic therapy to effect cures. Most of the other volunteers exhibited only a benign local lesion comparable to that of LVS vaccinees. Four of the 18 S1-11 vaccinees failed to develop agglutinins for Pasteurella tularensis, representing a higher percentage of "nontakes" than is routinely observed when LVS vaccine is administered. Approximately 4 months after vaccination, LVS and S1-11 vaccinees were permitted to inhale approximately 10,000 cells of strain SCHU S4. Nonvaccinated controls also received this dose. For LVS and S1-11 vaccinees that had developed agglutinins the protection afforded appeared comparable. Subsequent to the formidable aerogenic challenge of 10,000 organisms, 30 to 35% of these vaccinees became seriously ill and required antibiotic therapy in contrast to 95% of the nonvaccinated group.

In summary, a preliminary screening of several new attenuated mutants of P. tularensis in the mouse and guinea pig indicated that S1-11, a derivative of strain SCHU, offered potential as a live vaccine strain. Live tularemia vaccine prepared from S1-11 was more virulent for the guinea pig than LVS vaccine but was well tolerated by the monkey when administered intracutaneously or by multiple puncture. It was at least as immunogenic as LVS vaccine for the white mouse and monkey. In man more variation in local reaction, development of agglutinins, and lymphadenopathy was observed with S1-11 vaccine than with LVS vaccine. Comparable protection against a formidable aerogenic challenge with strain SCHU S4 was afforded S1-11 and LVS vaccinees who had developed P. tularensis agglutinins. Thus, SCHU S1-11 live tularemia vaccine does not appear to offer any advantage over LVS vaccine for the immunization of man, and its relatively greater reactivity severely limits its usefulness.